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Abstract

OBJECTIVE: To investigate and compare the protective impact of the in situ formed salivary pellicle on enamel and dentine erosion caused by different acids at pH 2.6. **METHODS:** Bovine enamel and dentine samples were exposed for 120 min in the oral cavity of 10 healthy volunteers. Subsequently, enamel and dentine pellicle-covered specimens were extraorally immersed in 1 ml hydrochloric, citric or phosphoric acid (pH 2.6, 60 s, each acid n=30 samples). Pellicle-free samples (each acid n=10) served as controls. Calcium release into the acid was determined by atomic absorption spectroscopy. The data were analysed by two-way ANOVA and Tukey's test ($\alpha=0.05$). **RESULTS:** Pellicle-covered samples showed significantly less calcium loss compared to pellicle-free samples in all acid groups. The mean (SD) pellicle protection (% reduction of calcium loss) was significantly better for enamel samples [60.9 (5.3)] than for dentine samples [30.5 (5.0)], but revealed no differences among the acids. **CONCLUSION:** The efficacy of the in situ pellicle in reducing erosion was 2-fold better for enamel than for dentine. Protection of the pellicle was not influenced by the kind of acid when enamel and dentine erosion was performed at pH 2.6.

Impact of the in situ formed salivary pellicle on enamel and dentine erosion induced by different acids

Abstract

Objective: This study aimed to investigate and compare the protective impact of the in situ formed salivary pellicle on enamel and dentine erosion caused by different acids at pH 2.6.

Methods: Bovine enamel and dentine samples were exposed for 120 min in the oral cavity of ten healthy volunteers. Subsequently, enamel and dentine pellicle-covered specimens were extraorally immersed in either 1 mL hydrochloric, citric or phosphoric acid (pH: 2.6, 60 s, each acid: n = 30 samples). Pellicle-free samples (each acid: n = 10) served as controls. Calcium release into the acid was determined by atomic absorption spectroscopy. The data were analysed by two-way ANOVA and Tukey's test ($\alpha= 0.05$).

Results: Pellicle-covered samples showed significantly less calcium loss compared to pellicle-free samples in all acid groups. The mean (SD) pellicle protection (% reduction of calcium loss) was significantly better for enamel: 60.9 (5.3) than for dentine samples: 30.5 (5.0), but revealed no differences among the acids.

Conclusion: The efficacy of the in situ pellicle to reduce erosion was twofold better for enamel than for dentine. The protection of the pellicle was not influenced by the kind of acid, when enamel and dentine erosion was performed at pH 2.6.

Key words: enamel, dentine, pellicle, acid, erosion

Introduction

Saliva plays an important role in minimizing erosive tooth wear due to its buffering and remineralizing capacity as well as the ability to form a protective pellicle layer on dental hard tissues [1,2]. In recent years, numerous studies focused on the protective impact of the in situ formed acquired pellicle on enamel surfaces [3-6]. Even a short time incubation of enamel samples in the oral cavity was shown to be sufficient to create a salivary pellicle on enamel, which protects the underlying enamel from erosion to some extent [4]. Pellicle layers formed in situ for 0.5, 1 and 2h [3] or 2, 6, 12 and 24 h [4], respectively, did not differ considerably in their ability to reduce enamel demineralization. However, the acid resistance of the pellicle layer itself seems to be dependent on the formation time, since the 2h-pellicle was dissolved from the enamel surface more rapidly if compared to 6h-, 12h- and 24h-biofilm [4]. TEM analysis showed that pellicle layers were dissolved continuously during acid exposure, but even after 5 min exposure to 1% citric acid, a residual pellicle layer could be detected on the enamel surface [6].

One recent study also found a protective, but limited effect of the acquired pellicle on dentine surfaces [7]. The thickness of the demineralized dentine surface after 5 min acid contact was not different for pellicle-free and pellicle-covered samples, but the presence of the pellicle significantly reduced calcium loss of the dentin samples [7]. In contrast, Hara et al.[8] showed that an intraorally formed 2h-pellicle is not effective in reducing dentine softening induced by an excessive acid challenge (10-30 min, orange juice).

Compared to the enamel pellicle, only limited information about the in situ pellicle on dentine erosion is available. Moreover, as yet, the protective impact of the in situ formed 2h-pellicle on enamel and dentine erosion is not compared within one protocol using the same method for analysis of erosion. In a previous in-situ-study conducted over 14 days it was shown that saliva offered better protection against erosion for enamel than for dentine. Thereby,

significantly less enamel and dentine loss could be observed for samples stored in situ compared to storage in water or saliva in vitro [9]. Due to the different composition of dental hard tissues it might be assumed that the protective properties of the pellicle might vary for enamel and dentine erosion. As different acids vary in their ability to demineralize bovine enamel [10], it might be interesting to analyze the protective properties of the pellicle layer might vary not only between different dental hard tissues but also between different acids. Thus, this study aimed to analyze and compare the protection of the in situ formed pellicle on enamel and dentine erosion caused by hydrochloric, citric and phosphoric acid.

Material and Methods

Volunteers and determination of saliva parameters

Ethic approval for the study was granted by the institutional ethics committee (07/11). Ten healthy subjects (2 male, 8 female) aged between 21 and 66 years took part in the study after signing an informed, written consent. Inclusion and exclusion criteria are given in Table I. The subjects had stimulated and unstimulated salivary flow rates (mean (SD)) of 1.2 (0.2) and 0.55 (0.1) mL/min, respectively. Saliva stimulation was performed by chewing of Paraffin tablets (Orion Diagnostica, Espoo, Finland). The pH-value amounted to 7.6 (0.3) (stimulated saliva) and 7.1 (0.2) (unstimulated saliva) and was determined by a pH meter (pH-meter 691, Methrom, Herisau, Switzerland). The buffering capacity was evaluated by titration (0.1 M HCl, Impulsomat 614, Methrom, Herisau, Switzerland) to pH 5.7 and amounted to 0.1 (0.0) and 0.1 (0.1) mL 0.1 M HCl/mL saliva for stimulated and unstimulated saliva, respectively.

Preparation of enamel and dentine specimens

Each 120 circular enamel and dentine samples (3 mm in diameter) were prepared from freshly extracted, non-damaged bovine incisors, which were stored in 0.9% NaCl solution until used. For preparation of dentine cylinders, enamel was completely removed until dentine was just exposed. The samples were embedded in acrylic resin (Paladur, Heraeus Kulzer, Germany), and their labial surfaces were ground flat and polished with water-cooled carborundum discs (500 - grit, Water Proof Silicon carbide Paper, Stuers, Erkrat, Germany) thereby removing approximately 200 μm of the outermost layer as checked with a micrometer (Digimatic, Mitutoyo, Tokyo, Japan). Finally, the samples were sterilized by γ -radiation (12 kGy, 4 h, Paul Scherrer Institute, Villigen, Switzerland).

In situ pellicle formation

Ninty enamel and ninty dentine samples were used for the in situ pellicle formation. Custom-made acrylic devices of the upper jaw were provided with buccal recesses in the areas of left and right maxillary second premolars and first molars for attachment of the samples. Nine enamel and nine dentine specimens were randomly assigned to each volunteer. Each volunteer had to conduct six runs, wearing the maxillary appliance loaded with three randomly assigned samples. The position of each sample in the device was randomly determined for each subject. The devices were inserted in the oral cavity between 8.00 and 8.30 on six days in sequence. The samples stayed in the oral cavity for 120 min. The participants were instructed to refrain from consumption of any dietary products and oral hygiene treatment 1 h before insertion and while the appliances were in place. For standardization reasons, volunteers were instructed to use fluoridated toothpaste (Aronal, GABA, Switzerland) and manual toothbrushes (Paro M 43, Esro AG, Thalwil, Switzerland) for 7 days prior to and during the course of the experiment. Thereby, the volunteers were advised to brush their teeth twice daily in the morning and evening using the fluoridated toothpaste.

Erosive challenge and calcium analysis

After 120 min pellicle formation, the enamel and dentine samples were carefully removed from the devices and rinsed with distilled water for 10 s.

Three enamel and three dentine pellicle-covered specimens of each volunteer were immersed in either hydrochloric acid (pH 2.6, 0.0025 mol/L), citric acid (pH 2.6, 0.0125 mol/L) or phosphoric acid (pH 2.6, 0.0025 mol/L) for 60 s. The pH of the acid was checked daily prior to the experiment. Each sample was eroded in 1 mL of the respective acid in an Eppendorf tube, which was gently shaken (180° rotation, 60x/min) during sample incubation. For determination of calcium release of pellicle-free samples, each 10 enamel and 10 dentine samples without pellicle were demineralized with 1 ml hydrochloric acid, citric acid or phosphoric acid (pH: 2.6) for 60 s. The amount of calcium dissolved from the enamel and dentine surfaces by erosive treatment was analysed by atomic absorption spectroscopy (Model 2380, Perkin-Elmer, Norwalk, CT) at 422.7 nm. The spectroscope was calibrated by calcium standard solutions. As phosphate might depress the sensitivity for calcium, 0.25% strontium chloride was added to the sample solutions to control these interferences.

In a previous study [11], the calcium release of pellicle-covered enamel specimens into distilled water and pellicle-covered acrylic resin specimens into hydrochloric acid was measured to analyse whether pellicle itself provides a reservoir of calcium ions. For both conditions, the calcium release was below the detection limit.

Statistical analysis

Mean calcium loss ($\mu\text{g Ca/ml acid}$) of pellicle-free and pellicle-covered enamel and dentine samples after treatment with hydrochloric, phosphoric or citric acid was calculated. The data

were statistically analyzed by two-way ANOVA, separately for the dental substrates, considering both the presence of pellicle and the kind of acid as independent variables. Among-group comparisons were made by Tukey's tests.

The mean percentage reduction of calcium loss for the enamel and dentine groups due to the presence of pellicle was calculated for each volunteer separately by comparison with the mean calcium loss in the respective pellicle-free group. Two-way ANOVA followed by Tukey's test were applied to analyze possible differences between enamel and dentine protection and among the different acids. Finally, mean calcium loss (% of the respective pellicle-free group) from the 3 different acids was integrated to compare the overall enamel or dentine protection by the presence of the salivary pellicle. The level of significance was set at $\alpha = 0.05$.

The analysis was performed with the software Graph Pad Prism 4 (San Diego, CA, USA).

Results

The presence of the salivary pellicle significantly reduced the amount of calcium loss compared to pellicle-free samples in all enamel and dentine groups (Figures 1 and 2, Table II). For enamel samples, two-way ANOVA revealed a significant difference ($p < 0.0001$) between the conditions "pellicle-free" and "pellicle-covered" as well as among the different acids. The interaction between the criteria (presence of pellicle and type of acid) was significant ($p = 0.007$). Pellicle-covered samples showed no significant differences between the different acid groups. In contrast, pellicle-free samples showed significantly higher calcium loss by erosion with citric acid compared to hydrochloric and phosphoric acid (Figure 1).

For dentine specimens, two-way ANOVA revealed a significant difference ($p = 0.0000$) between the conditions "pellicle-free" and "pellicle-covered" as well as among the acids. The interaction between the criteria (presence of pellicle and type of acid) was not significant ($p = 0.14$). Independently of the presence of the pellicle, citric acid caused significantly higher

calcium loss than hydrochloric and phosphoric acid, which in turn were not significantly different (Figure 2).

The analysis of pellicle protection (% reduction of calcium loss) revealed a significant difference between enamel and dentin protection ($p < 0.0001$), but not among the acids ($p = 0.25$). The interaction between these criteria (kind of dental hard tissue and type of acid) was not significant ($p = 0.18$). Independently of the acid, the protection of calcium loss was significantly better for the enamel pellicle than for the dentine pellicle (Table II).

Discussion

The results of this study show that pellicle is effective to reduce enamel and dentine erosion caused by different acids, but that the protective capacity of the acquired pellicle is approximately twofold better for enamel compared to dentine.

Bovine hard tissues were used as substitute for human teeth as done in several studies before [4,7,8]. However, although it was found that bovine and human enamel or dentine samples, respectively, vary in their susceptibility to erosion [12,13], there is no evidence that they also differ substantially in the formation or structure of the pellicle. Hove et al. [14] demonstrated that the effects of an in vitro formed pellicle on erosion of human and bovine enamel were not significantly different.

As recent studies of Hannig et al. [3] showed no difference in the protective capacity of pellicle formed in situ between 2 and 24 h or 2 h and 3 min, respectively, the samples were exposed to the oral cavity for 2 h to allow for pellicle formation. However, with regard to previous studies using a pellicle formation time of more than 2 h, it has to be taken into consideration that the structure and composition of 2-h salivary pellicle is different to a “matured” pellicle of several hours or days [15,16]. Similar to the erosion protective capacity of dental plaque [17], it is most frequently credited for its role in the caries process by harbouring cariogenic bacteria. As a previous study found the calcium release from the

pellicle itself to be below the detection limit [11], the mineral content of saliva might not affect the protective capacity of the pellicle.

Principally, the results of the present study confirm previous findings on the protective impact of the in situ formed salivary pellicle. Hannig et al.[3,4] found a reduction of calcium loss of 59.8 to 77.5 % for enamel samples eroded by 1% citric acid for 60 s. The overall protection of dentine (30.5 %), obtained by integrating the results of the three different acids, is in accordance to the results of the study by Hannig et al. [7], which showed a reduction of calcium erosion of about 27 % after treatment with hydrochloric acid (pH: 2.3, 5 min).

However, the design of the present study allows for direct comparison of the pellicle effects on erosion of enamel and dentine. To our knowledge this is the first study comparing the protective effect of the in situ formed salivary pellicle on short-term erosion by different acids of enamel and dentine in one protocol. It might be supposed that differences in the enamel or dentine structure might affect the composition or adsorption of the acquired pellicle [18] and, thus, the protective efficacy of the pellicle, even though previous studies showed that the substrate itself had only little impact on the pellicle formation and the presence and activity of enzymes [19-21]. The anti-erosive potential of the enamel salivary pellicle is attributed to its ability to act to some extent as a diffusion barrier as well as a semipermeable membrane [4]. From the limited potential of the dentine pellicle to protect the underlying dentine surface against erosion, it was assumed that the in situ formed 2 h dentinal pellicle mainly acts as an ion permeable network rather than a diffusion barrier [7]. Besides, Hara et al. [22] suggested that the higher porosity and solubility of dentine compared to enamel lead to a faster demineralization, which might prevent the pellicle to act as a protective barrier.

In accordance with the present study, Wetton et al. [23] also found that an in vitro formed salivary pellicle offered proportionately greater protection to enamel (44 %) than to dentine (14 %). Due to these results it was speculated that saliva penetrates the tubule system of dentine to produce not only a pellicle layer on the surface but a meniscus of viscous liquid at

the tubule orifices. Further studies are necessary to analyse possible differences in the composition and structure of the acquired pellicle on enamel and dentine, which might be responsible for the different protection. However, it might be not excluded that the protective capacity of the pellicle and the difference between enamel and dentine protection might be less evident under severe erosive conditions as used by Hara et al.[8].

In the present study, brief erosion of the samples was performed to simulate the clinical situation during the consumption of acidic drinks or the exposure of gastric acid in the oral cavity due to reflux or vomiting. Thus, citric acid and phosphoric acid were chosen as they are typically found in soft drinks (e.g. Sprite, Coke), and hydrochloric acid was selected as it is the main acid in gastric juice. The acids were applied at a standardized pH of 2.6, which is in the range of the pH levels typically found in erosive drinks and beverages [24]. Generally, citric acid was found to exhibit a greater erosivity than hydrochloric and phosphoric acid. This was also demonstrated by previous studies, which found citric acid to be more erosive than hydrochloric acid or phosphoric acid on enamel and dentine surfaces [10,25,26]. The greater erosive effect of citric acid might be related to its ability to form chelating complexes with calcium. Moreover, differences in their specific interaction with hydroxyapatite might influence the erosive potential of different mono-, di- and tri-carboxylic acids [10,26-28].

As the protective capability of the pellicle was not different among the different acids, it might be assumed that the partial dissolution or removal of the pellicle layer by the acids is similar for all enamel or dentine groups, respectively, and mainly dependent on the proton concentration. However, in further studies it has to be evaluated if the pellicle protection varies with different pH values of the acids.

In conclusion, the in situ pellicle is more effective in reducing enamel than dentine erosion, but, at pH 2.6, the protective capability of the pellicle is not dependent upon the acid applied.

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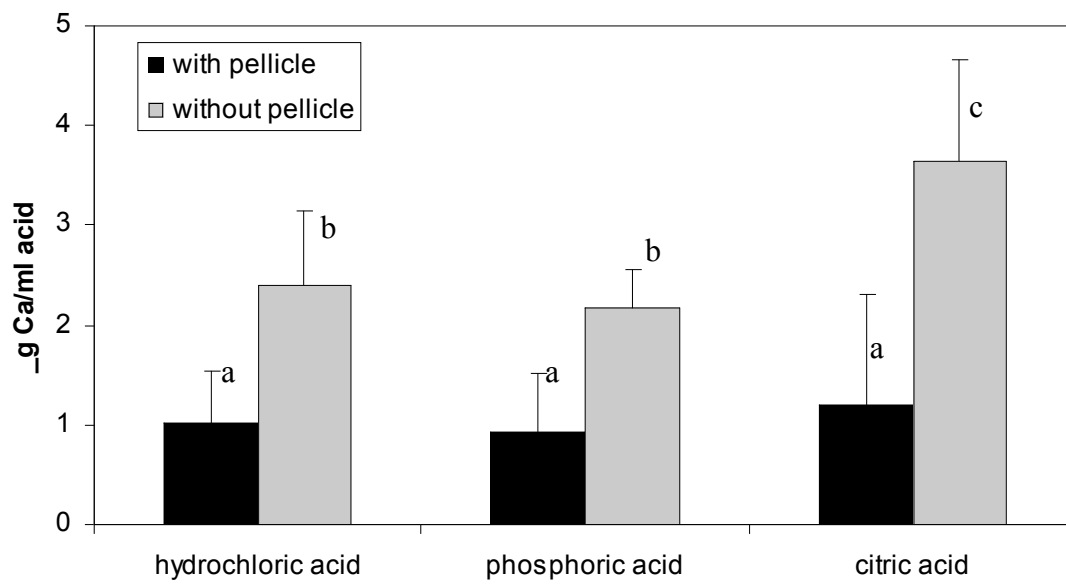


Figure 1

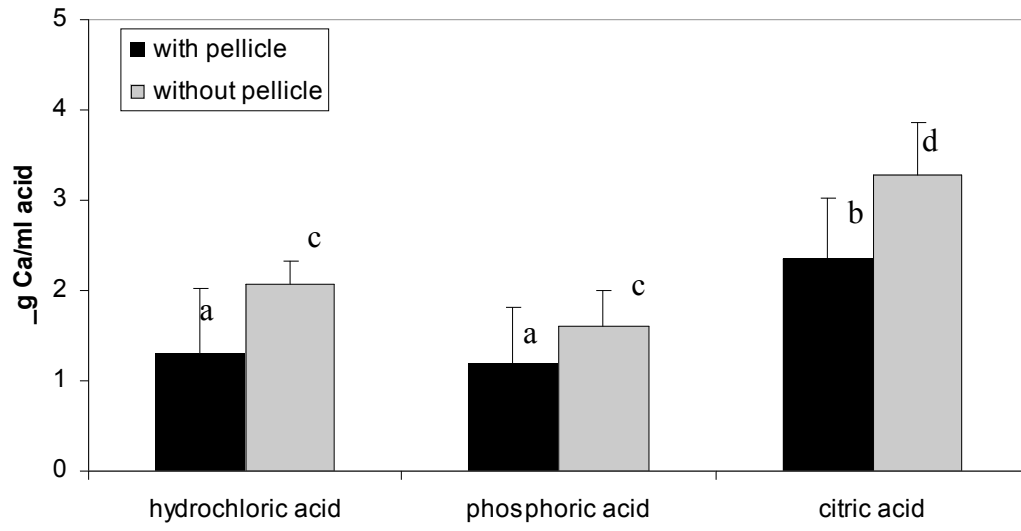


Figure 2

Legends to Figures

Figure 1:

Mean (SD) calcium loss ($\mu\text{g Ca/mL acid}$) in pellicle-covered and pellicle-free enamel samples. For all acid groups, pellicle-covered samples exhibited significantly less calcium loss than pellicle-free samples. Within pellicle-covered or pellicle-free samples, respectively, groups marked with the same letter were not significantly different.

Figure 2:

Mean (SD) calcium loss ($\mu\text{g Ca/mL acid}$) in pellicle-covered and pellicle-free dentine samples. For all acid groups, pellicle-covered samples exhibited significantly less calcium loss than pellicle-free samples. Within pellicle-covered or pellicle-free samples, respectively, groups marked with the same letter were not significantly different.

Tab. I

Inclusion and exclusion criteria for participation in the study.

Tab.II

Mean (SD) reduction of calcium loss in percent by the 120 min pellicle in enamel and dentine samples.

Inclusion criteria	Exclusion criteria
Male or female, > 18 years old	Use of fixed or removable orthodontic appliances
Classified as healthy	General/systemic illness
Mean stimulated saliva flow rate ≥ 1 mL/min	Hyposalivation
	Pregnancy or breastfeeding,

Table I

Type of acid	Substrate (mean (SD) % reduction of Ca loss)	
	Enamel	Dentine
Hydrochloric acid	58.0 (12.6)	36.2 (22.4)
Phosphoric acid	57.7 (15.1)	26.7 (19.3)
Citric acid	67.1 (17.7)	28.5 (12.4)
Mean (SD)	60.9 (5.3)	30.5 (5.0)

Table II